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In vitro variation in antibacterial activity plant extracts on *Glaucium elegans* and saffron *(Crocus sativus)*

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Abstract

The increase in antibiotic resistance has resulted in decreasing number active antimicrobial agents available to treat infections by multi-drug resistant (MDR) bacteria. The aim of this study was to determine the antimicrobial activity of the extracts of *Glaucium elegans* and Saffron *(Crocus sativus)* onios plant species against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritidis*, *Bacillus anthracis* and *Proteus* by disc diffusion method. The methanol extract of *G. elegans* was found to have a significant antibacterial efficiency ($p \le 0.05$) as compared to the methanol extract of onios plant. These finding pinpoint the efficiency of these extracts to inhibit microbial growth. The bactericidal activity described here represents an added safety value for *G. elegans* possesses the significant antibacterial activity.

Introduction

For over thousands of years now, natural plants have been seen as a valuable source of medicinal agents with proven potential of treating infectious diseases and with lesser side effects compared to the synthetic drug agents (Nagham, 2011). Different types of medicinal plants for their anti-oxidant and antimicrobial potential are *Amberboa divaricata* (Iqbal et al., 2014), *Artemisia annua* (Zhang et al., 2012), *Asparagus laricinus* (Ntsoe-linyane and Mashele, 2014), *Asparagus racemesus* (Shah et al., 2014), *Berberis jaeschkeana* (Alamzeb et al., 2013), *Buddleja polystachya* (Fawzy et al., 2013), *Leucas clarkei* (Das et al., 2012), *Phyllostachys edulis* (Shen et al., 2012), *Pithecellobium jiringa* (Bakar et al., 2012), *Rhododendron arboretum* (Nisar et al., 2013), *Sarcochlamys pulcherrima* (Mazumder et al., 2014).

The genus *Glaucium* Mill. Belongs to *Papaveraceae* family and consists of 25 species, annuals, biennials and perennials. They grow in middle and South West Asia and Europe (Burnie et al., 2004). This genus consists of herbaceous plants. Cullen (1966) in Flora Iranica area recognized 11 species, 10 of which occurring in Iran, one including two subspecies. Mobayen (1984) descrybed two new species, namely *G. elegantissimum* and *G. mathiolifolium*. Later on Mobayen (1985) in Flora of Iran, vascular plants apparently recognized 14 species in Iran, several of them including subspecific taxa (Gran and Sharifnia, 2008).

Saffron is the most expensive spice in the world and consists of the dried stigmas of *Crocus sativus* L. By far the most important producer is Iran, followed by Greece, Morocco, India, Spain and Italy. Saffron is greatly appreciated for its abilities of coloring and flavouring, and for its aromatic strength. These properties are basically related to its contents of picrocrocin, safranal and crocins (Rafael et al., 2011). Picrocrocin is the glycoside precursor of safranal (2, 6, 6-trimethyl-1, 3 -cyclohexadiene-1-carboxaldehyde), which is in turn the most abundant of the volatile compounds responsible for the aroma of this spice (Maggi et al., 2009).

Spices have been used since ancient times to hide spoilage in foods. Nonetheless, as with many other agricultural products, spices can suffer microbial contamination and their additions to ready-to eat foods can result in the proliferation of bacterial pathogens (Vij et al., 2006; Sagoo et al., 2009). The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains (Patterson, 2000). The antimicrobial compounds from plants may inhibit bacteria by a different mechanism than the presently used antibiotics and may have clinical value in treatment of resistant microbial strains (5.whi). Because of the side effects and bacteria resistance against the antibiotics, the scientist developed new drugs from natural sources such as plants, which have been extensively used as alternative treatment for disease (Sumitra and Yogesh, 2010; Manoj et al., 2010).

The purpose of the present study was to investigate antimicrobiyal properties of *G. elegans* and Saffron onion. In this paper we report the results of such studies in order to orient future investigations towards the finding of new, potent and safe antimicrobial compounds.

Materials and Methods

Plant materials

G. elegans and Saffron onion were collected from different natural habitats of the country. The onion of Saffron and Leaves and twigs of *G. elegans* separated and dried in dark at room temperature. In order to extract content of 12 g fresh sample prepared with distilled water and poured into an Erlenmeyer flask and heated for 30 min at 100°C was boiled. The extracts were filtered using Whitman Strainer paper and then in dry heat of 55°C for 24 hours were exposed to the extract powder is produced. One gram of the powder, each of which was dissolved in 10 mL of deionized water. Different concentration (250, 750 and 1500 µg/ disc) and the extracts were prepared calculated.

Bacterial strains and culture media

Five pathogenic bacteria including five strains of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, *Bacillus anthracis* and *Proteus* were used for the bioassay study. The pure strain was identified and obtained from Microbiology and Biotechnology Laboratory, Department of Islam Islamic Azad University, Shahrekord Branch, Shahrekord, Iran. All cultures were incubated at 37°C in aerobic conditions. The test organisms were transferred to the agar slants from the supplied pure cultures with the help of an inoculating loop in an aseptic condition. The inoculated slants were then incubated at 37.5°C for 24 hours to ensure the growth of test organisms. These fresh cultures were used for the sensitivity test.

Preparation of discs

Three types of discs were prepared for antibacterial screening. Sterilized (BBL, USA) filter paper discs (5 mm in diameter) were taken in blank Petri dishes. Sample solution of desired concentration (10 µL/ disc) was applied on the discs with the help of a micropipette in an aseptic condition. These were used to compare the antibacterial activity of test material. In our investigation, Gentamicin (1.3 µg/disc) was used as standard disc. These were prepared by using identical filter paper (5 mm diameter) and same volume of residual solvent in the same condition. These were used as negative control to ensure that the residual solvent and the filter paper themselves was not active.

Placement of the discs and incubation

The dried crude extract separate discs and standard discs were placed on the solidified agar plates seeded with the test organisms. The plates were kept in a refrigerator at 4°C for 24 hours in order to provide sufficient time to diffuse the antibiotics into the medium. Then the plates were incubated at 37.5°C for 24 hours in an incubator.

Measurement of the zones of inhibition

After incubation, the antibacterial activities of the test samples were determined by measuring the diameter of inhibitory zones (mm) with a transparent scale.

Statistical analysis

The data were analyzed using Analysis of Variance (ANOVA) test. A one-way ANOVA was performed on all results. Probabilities of less than 0.05 were considered statistically significant. Differences between means were determined using Duncan compromise test. The software SPSS (Ver. 17) was used to conduct all the statistical analysis.

Results and Discussion

The tests for antibacterial effect establish of vegetal extracts revealed that most of the extracts are representing an important source of substances with antimicrobial activity. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents (Kone et al., 2004).

The antimicrobial activity of extract having different concentrations (250, 750 and 1500 µg/disc), was tested against five pathogenic bacteria. Gentamicin disc (1.25 µg/disc) was used for comparing the antibacterial activity. Tables I represents the inhibition zone, found by the activity of extracts on micro-organisms tested and compared with gentamicin. At 250 µg/disc dosage extract of *G. elegans* showed 1, 2 and 1 mm zone of inhibition against *E. coli, Proteus* and *B. anthracis* respectively.

Table I

Inhibition areas diameter of the tested plant essential extracts on microorganisms tested and compared with gentamicin

	Concentration (µg/disc)	*B. anthracis*	*S. enteritidis*	*S. aureus*	*Proteus*	*E. coli*
Glaucium elegans	250	1.0 ± 0.1	-	-	2 ± 0.2	1 ± 0.3
Glaucium elegans	750	17 ± 0.2	9 ± 0.1	12 ± 0.6	18 ± 0.3	10 ± 0.4
Glaucium elegans	1500	21 ± 0.8	13 ± 01	22 ± 0.5	23 ± 0.7	13 ± 0.4
Saffron onion	250	-	-	-	-	-
Saffron onion	750	-	-	-	-	-
Saffron onion	1500	-	-	-	-	-
Gentamcin	1.25	28 ± 0.7	25 ± 0.5	26 ± 0.4	17 ± 0.6	27 ± 0.5

However no antimicrobial activity was observed against *S. aureus* and *S. enteritidis* the same concentration. At 750 µg/disc Concentration of *G. elegans*, the zone of inhibition against the same bacteria was 10, 18, 12, 9 and 17 mm respectively against *E. coli, Proteus*, *S. aureus*, *S. enteritidis* and *B.anthracis* respectively. In case of highest dose of 1500 µg/disc dosage extract of *G. elegans*, the zones of inhibition were found to be 13, 23, 22, 13 and 21 mm respectively (Figure 1).

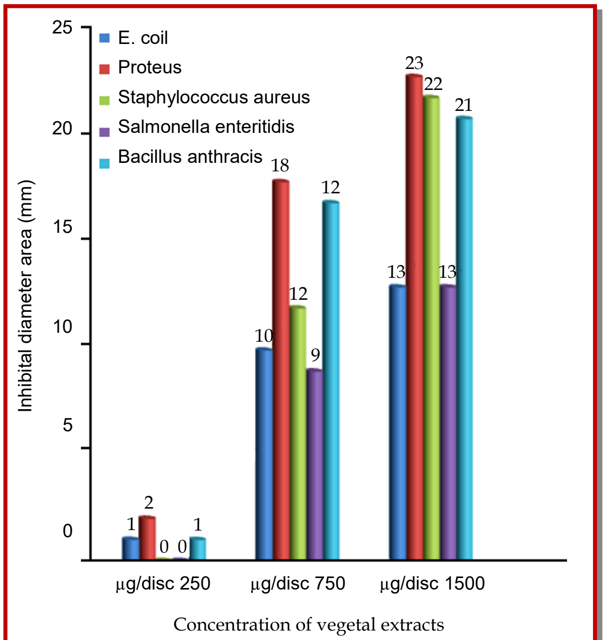

Figure 1: Antimicrobial activity extract of *G. elegans*

At all concentrations dosage extract of saffron onion, no antimicrobial activity was observed against bacterias. But the standard antibiotic disc gentamicin (1.3 µg/disc) showed the zone of inhibition against five bacteria such as *E. coli, Proteus*, *S. aureus*, *S. enteritidis* and *B. anthracis*. The zones of inhibitions were 27, 19, 26, 25 and 28 mm respectively.

Tested essential extracts most antimicrobial effect of the *Proteus* strains including the strains with re-sistance to antibiotics. It can be concluded that *G. elegans* has a wide range of antibacterial activity as medicines. Also in research on extract of *Salvia mirzayanii* demonstrated antimicrobial effect against *S. aureus* and *E. colli* (Moshafi et al., 2004). In other study assay, the methanol extract of red variety was found to have a significant antibacterial effect against *S. aureus, E. colli* (Krishna et al., 2012).

In summary, *G. elegans* extracts could be an important source of antibiotics compounds potentially active against bacterias.

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